

**METHOD 531.1. MEASUREMENT OF N-METHYLCARBAMOYLOXIMES AND N-METHYLCARBAMATES
IN WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POST COLUMN
DERIVATIZATION**

Revision 3.1

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METHOD 531.1

MEASUREMENT OF N-METHYLCARBAMOYLOXIMES AND N-METHYLCARBAMATES IN WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POST COLUMN DERIVATIZATION

1. SCOPE AND APPLICATION

- 1.1 This is a high performance liquid chromatographic (HPLC) method applicable to the determinations of certain N-methylcarbamoxyloximes and N-methylcarbamates in ground water and finished drinking water. The following compounds can be determined using this method:

<u>Analyte</u>	<u>Chemical Abstract Services Registry Number</u>
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Baygon	114-26-1
Carbaryl	63-25-2
Carbofuran	1563-66-2
3-Hydroxycarbofuran	16655-82-6
Methiocarb	2032-65-7
Methomyl	16752-77-5
Oxamyl	23135-22-0

- 1.2 This method has been validated in a single laboratory and estimated detection limits (EDLs) and method detection limits (MDLs) have been determined for the analytes above. Observed detection limits may vary between ground waters, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.
- 1.3 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.
- 1.4 When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications should be confirmed by at least one additional qualitative technique (1).

2. SUMMARY OF METHOD

- 2.1 The water sample is filtered and a 400- μ L aliquot is injected into a reverse phase HPLC column. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed with 0.05 N sodium hydroxide (NaOH) at 95°C. The methyl amine formed during hydrolysis is

reacted with o-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative which is detected by a fluorescence detector (2). Analytes are quantitated using procedural standard calibration (Sect. 3.14)

3. DEFINITIONS

- 3.1 INTERNAL STANDARD -- A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.
- 3.2 SURROGATE ANALYTE -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.
- 3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.4 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.
- 3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.6 FIELD REAGENT BLANK (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.
- 3.7 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) -- A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

- 3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.
- 3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.10 STOCK STANDARD SOLUTION -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.
- 3.11 PRIMARY DILUTION STANDARD SOLUTION -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.
- 3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.13 QUALITY CONTROL SAMPLE (QCS) -- A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.
- 3.14 PROCEDURAL STANDARD CALIBRATION -- A calibration method where aqueous calibration standards are prepared and processed (e.g. purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

4. INTERFERENCES

- 4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead

to discrete artifacts or elevated baselines in liquid chromatograms. Specific sources of contamination have not been identified. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned.(3) Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric glassware. Thermally stable materials might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

4.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. A preventive technique is between-sample rinsing of the sample syringe and filter holder with two portions of reagent water. After analysis of a sample containing high concentrations of analytes, one or more laboratory reagent blanks should be analyzed.

4.3 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from source to source, depending upon the water sampled. Analyte identifications must be confirmed. Positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used; e.g., mass spectrometry, or the use of a second chromatography column. A suggested alternative column is described in Sect. 6.6.3.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file

of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (4-6) for the information of the analyst.

5.2 WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 SAMPLING EQUIPMENT

6.1.1 Grab sample bottle -- 60-mL screw cap vials (Pierce No. 13075 or equivalent) and caps equipped with a PTFE-faced silicone septa (Pierce No. 12722 or equivalent). Prior to use, wash vials and septa as described in Sect. 4.1.1.

6.2 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3 FILTRATION APPARATUS

6.3.1 Macrofiltration -- To filter derivatization solutions and mobile phases used in HPLC. Recommend using 47 mm filters (Millipore Type HA, 0.45 μ m for water and Millipore Type FH, 0.5 μ m for organics or equivalent).

6.3.2 Microfiltration -- To filter samples prior to HPLC analysis. Use 13 mm filter holder (Millipore stainless steel XX300/200 or equivalent), and 13 mm diameter 0.2 μ m polyester filters (Nuclepore 180406 or equivalent).

6.4 SYRINGES AND SYRINGE VALVES

6.4.1 Hypodermic syringe -- 10-mL glass, with Luer-Lok tip.

6.4.2 Syringe valve -- 3-way (Hamilton HV3-3 or equivalent).

6.4.3 Syringe needle -- 7 to 10-cm long, 17-gauge, blunt tip.

6.4.4 Micro syringes -- various sizes.

6.5 MISCELLANEOUS

6.5.1 Solution storage bottles -- Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw cap.

6.5.2 Helium, for degassing solutions and solvents.

6.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC)

- 6.6.1 HPLC system capable of injecting 200- to 400- μ L aliquots, and performing binary linear gradients at a constant flow rate. A data system is recommended for measuring peak areas. Table 1 lists retention times observed for method analytes using the columns and analytical conditions described below.
- 6.6.2 Column 1 (Primary column) -- 150 mm long x 3.9 mm I.D. stainless steel packed with 4 μ m NovaPak C18. Mobil Phase is established at 10:90 methanol:water, hold 2 min., then program as a linear gradient to 80:20 methanol:water in 25 min. Alternative columns may be used in accordance with the provisions described in Sect. 9.4.
- 6.6.3 Column 2 (Alternative column)* -- 250 mm long x 4.6 mm I.D. stainless steel packed with 5 μ m Beckman Ultrasphere ODS. Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol:water to 100 % methanol in 32 min. Data presented in this method were obtained using this column. * Newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.
- 6.6.4 Column 3 (Alternative column) -- 250 mm long x 4.6 mm I.D. stainless steel packed with 5 μ m Supelco LC-1. Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol:water to 100 % methanol in 32 min.
- 6.6.5 Post column reactor -- Capable of mixing reagents into the mobile phase. Reactor should be constructed using PTFE tubing and equipped with pumps to deliver 0.1 to 1.0 mL/min of each reagent; mixing tees; and two 1.0-mL delay coils, one thermostated at 95°C (ABI URS 051 and URA 100 or equivalent).
- 6.6.6 Fluorescence detector -- Capable of excitation at 330 nm (nominal) and detection of emission energies greater than 418 nm. A Schoffel Model 970 fluorescence detector was used to generate the validation data presented in this method.

7. REAGENTS AND STANDARDS -- WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

- 7.1 REAGENT WATER -- Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Reagent water used to generate the validation data in this method was distilled water obtained from the Magnetic Springs Water Co., 1801 Lone Eagle St., Columbus, Ohio 43228.

7.2 METHANOL -- Distilled-in-glass quality or equivalent.

7.3 HPLC MOBILE PHASE

7.3.1 Water -- HPLC grade (available from Burdick and Jackson).

7.3.2 Methanol -- HPLC grade. Filter and degas with helium before use.

7.4 POST COLUMN DERIVATIZATION SOLUTIONS

7.4.1 Sodium hydroxide, 0.05 N -- Dissolve 2.0 g of sodium hydroxide (NaOH) in reagent water. Dilute to 1.0 L with reagent water. Filter and degas with helium just before use.

7.4.2 2-Mercaptoethanol (1+1) -- Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap. Store in hood (CAUTION -- stench).

7.4.3 Sodium borate (0.05 N) -- Dissolve 19.1 g of sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in reagent water. Dilute to 1.0 L with reagent water. The sodium borate will completely dissolve at room temperature if prepared a day before use.

7.4.4 OPA reaction solution -- Dissolve 100 ± 10 mg of o-phthalaldehyde (mp 55-58°C) in 10 mL of methanol. Add to 1.0 L of 0.05 N sodium borate. Mix, filter, and degas with helium. Add 100 μL of 2-mercaptoethanol (1+1) and mix. Make up fresh solution daily.

7.5 MONOCHLOROACETIC ACID BUFFER (pH3) -- Prepare by mixing 156 mL of 2.5 M monochloroacetic acid and 100 mL 2.5 M potassium acetate.

7.6 4-BROMO-3,5-DIMETHYLPHENYL N-METHYLCARBAMATE (BDMC) -- 98% purity, for use as internal standard (available from Aldrich Chemical Co.).

7.7 STOCK STANDARD SOLUTIONS (1.00 $\mu\text{g}/\mu\text{L}$) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

7.7.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

- 7.7.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at room temperature and protect from light.
- 7.7.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.
- 7.8 INTERNAL STANDARD SOLUTION -- Prepare an internal standard fortification solution by accurately weighing approximately 0.0010 g of pure BDMC. Dissolve the BDMC in methanol and dilute to volume in a 10-mL volumetric flask. Transfer the internal standard fortification solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Addition of 5 μ L of the internal standard fortification solution to 50 mL of sample results in a final internal standard concentration of 10 μ g/L. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem. Note: BDMC has been shown to be an effective internal standard for the method analytes, but other compounds may be used, if the quality control requirements in Sect. 9 are met.
- 7.9 LABORATORY PERFORMANCE CHECK SOLUTION -- Prepare concentrate by adding 20 μ L of the 3-hydroxycarbofuran stock standard solution, 1.0 mL of the aldicarb sulfoxide stock standard solution, 200 μ L of the methiocarb stock standard solution, and 1 mL of the internal standard fortification solution to a 10-mL volumetric flask. Dilute to volume with methanol. Thoroughly mix concentrate. Prepare check solution by placing 100 μ L of the concentrate solution into a 100-mL volumetric flask. Dilute to volume with buffered reagent water. Transfer to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem.

8. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 8.1 Grab samples must be collected in glass containers. Conventional sampling practices (8) should be followed; however, the bottle must not be prerinsed with sample before collection.
- 8.2 SAMPLE PRESERVATION/PH ADJUSTMENT -- Oxamyl, 3-hydroxycarbofuran, aldicarb sulfoxide, and carbaryl can all degrade quickly in neutral and basic waters held at room temperature. (6,7) This short term degradation is of concern during the time samples are being shipped and the time processed samples are held at room temperature in autosampler trays. Samples targeted for the analysis of these three analytes must be preserved at pH 3. The pH adjustment also minimizes analyte biodegradation.
- 8.2.1 Add 1.8 mL of monochloroacetic acid buffer to the 60-mL sample bottle. Add buffer to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site.

- 8.2.2 If residual chlorine is present, add 80 mg of sodium thio-sulfate per liter of sample to the sample bottle prior to collecting the sample.
- 8.2.3 After sample is collected in bottle containing buffer, seal the sample bottle and shake vigorously for 1 min.
- 8.2.4 Samples must be iced or refrigerated at 4°C from the time of collection until analysis is begun. Although preservation results of up to 28 days indicate method analytes are not labile in water samples when sample pH is adjusted to 3 or less, and samples are shipped and stored at 4°C, analyte lability may be affected by the matrix. Therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

9. QUALITY CONTROL

- 9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, monitoring internal standard peak area or height in each sample and blank (when internal standard calibration procedures are being employed), analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks and QC samples. A MDL for each analyte must also be determined.
- 9.2 LABORATORY REAGENT BLANKS. Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.
- 9.3 INITIAL DEMONSTRATION OF CAPABILITY.
 - 9.3.1 Select a representative concentration, about 10 times EDL, or a concentration that represents a medium concentration calibration standard for each analyte. Prepare a primary dilution standard (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 50 μ L of the concentrate to each of four to seven 50-mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Sect. 11.
 - 9.3.2 For each analyte the recovery value for all of these samples must fall in the range of $\pm 20\%$ of the fortified amount, and the RSD of the measurements must be 20% or less. For those compounds that meet the acceptance criteria, performance is judged acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be

repeated using four samples until satisfactory performance has been demonstrated.

9.3.3 To determine the MDL, analyze a minimum of 7 LFBs prepared at a low concentration. Use the concentrations in Table 3 as a guide, or use calibration data to estimate a concentration that will yield a peak with a signal to noise ratio of approximately 5. Analyze the 7 replicates as described in Sect. 11, and on a schedule that results in the analyses being conducted over several days. Calculate the mean accuracy and standard deviation for each analyte. Calculate the MDL using the equation given in Table 3.

9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.

9.4 The analyst is permitted to modify HPLC columns, HPLC conditions, and internal standards to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.

9.5 ASSESSING THE INTERNAL STANDARD

9.5.1 When using the internal standard calibration procedure, the analyst must monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standard's IS response by more than 30%.

9.5.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot.

9.5.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.5.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the sample should be repeated beginning with Sect. 11, provided a duplicate sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.5.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

9.5.3.1 If the check standard provides a response for the IS within 20% of the predicted value, then follow procedures itemized in Sect. 9.5.2 for each sample failing the IS response criterion.

9.5.3.2 If the check standard provides a response for the IS which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Sect. 10.

9.6 ASSESSING LABORATORY PERFORMANCE - LABORATORY FORTIFIED BLANKS

9.6.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples analyzed within a 24-h period) whichever is greater. The fortification concentration of each analyte in the LFB should be 10 times EDL or a concentration in the middle of the calibration range. Calculate accuracy as percent recovery (X_i). If the recovery of any analyte falls outside the control limits (see Sect. 9.6.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.6.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 9.3.2. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery (\bar{X}) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

$$\begin{aligned}\text{UPPER CONTROL LIMIT} &= \bar{X} + 3S \\ \text{LOWER CONTROL LIMIT} &= \bar{X} - 3S\end{aligned}$$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should not exceed those established in Sect. 9.3.2.

9.6.3 If acceptable accuracy and method detection limits cannot be achieved, the problem must be located and corrected before further samples are analyzed. Data from all field samples analyzed since the last acceptable LFB should be considered suspect, and duplicate samples should be analyzed, if they are available, after the problem has been corrected. LFB results should be added to the on-going control charts to document data quality.

Since the calibration check sample in Sect. 10.2.4 and 10.3.3 and the LFB are made the same way and since procedural

standards are used, the sample analyzed here may also be used as a calibration check as described in those sections.

9.6.4 It is recommended that the laboratory periodically determine and document its detection limit capabilities for analytes of interest.

9.6.5 At least quarterly, analyze a QC sample from an outside source.

9.7 ASSESSING ANALYTE RECOVERY - LABORATORY FORTIFIED SAMPLE MATRIX

9.7.1 The laboratory must add a known concentration to a minimum of 5% of the routine samples or one sample per set, whichever is greater. The concentration should not be less than the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.6). Over time, samples from all routine sample sources should be fortified.

9.7.2 Calculate the percent recovery, P, of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.,:

$$P = 100 (X - b) / \text{fortifying concentration},$$

and compare these values to control limits appropriate for reagent water data collected in the same fashion. The value for P must fall in the range of 65%-135% of the fortified amount.

9.7.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.6), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.8 ASSESSING INSTRUMENT SYSTEM - LABORATORY PERFORMANCE CHECK SAMPLE -

Instrument performance should be monitored on a daily basis by analysis of the LPC sample. The LPC sample contains compounds designed to monitor instrument sensitivity, column performance (primary column) and chromatographic performance. LPC sample components and performance criteria are listed in Table 4. Inability to demonstrate acceptable instrument performance indicates the need for reevaluation of the instrument system. The sensitivity requirements are set based on the EDLs published in this method. If laboratory EDLs differ from those listed in this method,

concentrations of the LPC standard compounds must be adjusted to be compatible with the laboratory EDLs.

- 9.9 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10. CALIBRATION AND STANDARDIZATION

- 10.1 Establish HPLC operating parameters equivalent to those indicated in Sect. 6.6. The HPLC system may be calibrated using either the internal standard technique (Sect. 10.2) or the external standard technique (Sect. 10.3).

- 10.2 INTERNAL STANDARD CALIBRATION PROCEDURE. The analyst must select one or more internal standards similar in analytical behavior to the analytes of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. BDMC has been identified as a suitable internal standard.

- 10.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or more of the stock standards to a volumetric flask. Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. The lowest standard should represent analyte concentrations near, but above, their respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample, or should define the working range of the detector. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with buffered reagent water. To prepare buffered reagent water, add 10 mL of 1.0 M monochloroacetic acid buffer to 1 L of reagent water.

- 10.2.2 Analyze each calibration standard according to the procedure in Sect. 11. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each analyte and surrogate using Equation 1.

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \quad \text{Equation 1}$$

where:

- A_s = Response for the analyte to be measured.
- A_{is} = Response for the internal standard.
- C_{is} = Concentration of the internal standard $\mu\text{g/L}$.
- C_s = Concentration of the analyte to be measured $\mu\text{g/L}$.

10.2.3 If the RF value over the working range is constant (20% RSD or less), the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios (A_s/A_{is}) vs. C_s .

10.2.4 The working RF or calibration curve must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the concentration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than $\pm 20\%$, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

10.3 EXTERNAL STANDARD CALIBRATION PROCEDURE

10.3.1 Prepare calibration standards as described in Sect 10.2.1 omitting the use of an internal standard.

10.3.2 Starting with the standard of lowest concentration, analyze each calibration standard according to Sect. 11.2 and tabulate responses (peak height or area) versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range ($<20\%$ relative standard deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

10.3.3 The working calibration curve or calibration factor must be verified on each working day as described in Sect. 10.2.4.

10.4 Verify calibration standards periodically, recommend at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration (Sect. 10.2.4).

11. PROCEDURE

11.1 PH ADJUSTMENT AND FILTRATION

11.1.1 Add preservative to any samples (LFBs, LRBs, or calibration standards) not previously preserved (Sect. 8). Adjust the pH of the sample or standard to $\text{pH } 3 \pm 0.2$ by adding 1.5 mL of 2.5 M monochloroacetic acid buffer to each 50 mL of sample. This step should not be necessary if sample pH was adjusted during sample collection as a preservation precaution. Fill a 50-mL volumetric flask to the mark with the sample. Add 5 μL of the internal standard fortification solution (if the internal standard calibration procedure is being employed) and mix by inverting the flask several times.

11.1.2 Affix the three-way valve to a 10-mL syringe. Place a clean filter in the filter holder and affix the filter holder and the 7- to 10-cm syringe needle to the syringe valve. Rinse the needle and syringe with reagent water. Prewet the filter by passing 5 mL of reagent water through the filter. Empty the syringe and check for leaks. Draw 10 mL of sample into the syringe and expel through the filter. Draw another 10 mL of sample into the syringe, expel through the filter, and collect the last 5 mL for analysis. Rinse the syringe with reagent water. Discard the filter.

11.2 LIQUID CHROMATOGRAPHY

11.2.1 Sect. 6.6 summarizes the recommended operating conditions for the liquid chromatograph. Table 1 lists retention times observed using this method. Other HPLC columns or chromatographic conditions may be used if the requirements of Sect. 9 are met.

11.2.2 Calibrate or verify the system calibration daily as described in Sect. 10. The standards and samples must be in pH 3 buffered water.

11.2.3 Inject 400 μL of the sample. Record the volume injected and the resulting peak size in area units.

11.2.4 If the response for the peak exceeds the working range of the system, dilute the sample with pH 3 buffered reagent water and reanalyze.

11.3 IDENTIFICATION OF ANALYTES

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.3.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternate techniques, to help confirm peak identification, need to be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used; e.g., mass spectrometry (1), or the use of a second chromatography column. A suggested alternative column is described in Sect. 6.6.3.

12. CALCULATIONS

Determine the concentration of individual compounds in the sample using the following equation:

$$C_x = \frac{(A_x)}{(A_s)} \frac{(Q_s)}{(RF)},$$

where: C_x = analyte concentration in micrograms per liter;

A_x = response of the sample analyte;

A_s = response of the standard (either internal or external), in units consistent with those used for the analyte response;

RF = response factor (with an external standard, RF = 1, because the standard is the same compound as the measured analyte; with an internal standard RF is a unitless value);

Q_s = concentration of internal standard present or concentration of external standard that produced A_s , in micrograms per liter.

Use the multi-point calibration established in Section 10 for all calculations. Do not use the daily calibration verification data to quantitate analytes in samples.

13. METHOD PERFORMANCE

- 13.1 In a single laboratory, analyte recoveries from reagent water were used to determine analyte MDLs and EDLs and demonstrate method range. Analyte recoveries and standard deviation about the percent recoveries at one concentration are given in Table 3.
- 13.2 In a single laboratory, analyte recoveries from two standard synthetic ground waters were determined at one concentration level. Results were used to demonstrate applicability of the method to different ground water matrices. Analyte recoveries from the two synthetic matrices are given in Table 2.

14. REFERENCES

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5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
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***17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

TABLE 1. RETENTION TIMES FOR METHOD ANALYTES

Analyte	Retention Time ^(a) (minutes)		
	Primary ⁽¹⁾	Alternative ⁽²⁾	Alternative ⁽³⁾
Aldicarb sulfoxide	6.80	15.0	17.5
Aldicarb sulfone	7.77	15.2	12.2
Oxamyl	8.20	17.4	14.6
Methomyl	8.94	18.4	14.8
3-Hydroxycarbofuran	13.65	23.3	19
Aldicarb	16.35	27.0	21.4
Baygon	18.86	29.3	24.4
Carbofuran	19.17	29.6	23.4
Carbaryl	20.29	30.8	25.4
Methiocarb	24.74	34.9	28.6
BDMC	25.28	35.5	

^(a) Columns and analytical conditions are described in Sect. 6.6.

(1) Waters NovaPak C18

(2) Beckman Ultrasphere ODS

(3) Supelco LC-1

TABLE 2. SINGLE LABORATORY ACCURACY AND PRECISION FOR ANALYTES FROM REAGENT WATER AND SYNTHETIC GROUND WATERS^a

Analyte	Fortified Concentration μg/L	Reagent Water		Synthetic Water 1 ^d		Synthetic Water 2 ^e	
		R ^b	S _R ^c	R	S _R	R	S _R
Aldicarb	5.0	115	3.5	106	3.2	102	8.2
Aldicarb sulfone	10.	101	4.0	98	3.9	95	9.5
Aldicarb sulfoxide	10.	97	4.9	105	4.2	94	10.3
Baygon	5.0	106	3.2	96	4.8	97	5.8
Carbaryl	10.	97	5.8	94	4.7	104	10.4
Carbofuran	7.5	102	5.1	102	3.1	100	7.0
3-Hydroxycarbofuran	10.	102	4.1	98	4.9	101	10.1
Methiocarb	20.	94	1.9	102	4.1	112	3.4
Methomyl	2.5	105	4.2	98	3.9	105	9.5
Oxamyl	10.	100	4.0	97	2.9	102	10.2

^a Data corrected for amount detected in blank and represent the mean of 7-8 samples.

^b R = Average percent recovery.

^c S_R = Standard deviation of the percent recovery.

^d Corrected for amount found in blank; Absopure Nature Artesian Spring Water Obtained from the Absopure Water Company in Plymouth, Michigan.

^e Corrected for amount found in blank; reagent water fortified with fulvic acid at the 1 mg/L concentration level. A well-characterized fulvic acid, available from the International Humic Substances Society (associated with the United States Geological Survey in Denver, Colorado), was used.

TABLE 3. SINGLE LABORATORY ACCURACY, PRECISION, METHOD DETECTION LIMITS (MDLs), AND ESTIMATED DETECTION LIMITS (EDLs) FOR ANALYTES FROM REAGENT WATER

Analytes	Fortified $\mu\text{g/L}$	N ^a	Recovery %	RSD %	MDL ^b $\mu\text{g/L}$	EDL ^c $\mu\text{g/L}$
Aldicarb	1.0	8	107	7	0.22	1.0
Aldicarb sulfone	2.0	8	83	20	1.0	2.0
Aldicarb sulfoxide	2.0	8	47	21	0.59	2.0
Baygon	1.0	7	101	32	1.0	1.0
Carbaryl	2.0	8	97	23	1.3	2.0
Carbofuran	1.5	7	90	12	0.52	1.5
3-Hydroxycarbofuran	2.0	8	108	29	1.9	2.0
Methiocarb	4.0	8	82	19	1.9	4.0
Methomyl	0.50	7	102	18	0.29	0.50
Oxamyl	2.0	8	82	17	0.86	2.0

^a N = Number of replicates

^b MDL = $S \cdot t_{(n-1, 1-\alpha)} = 0.99$

where:

$t_{(n-1, 1-\alpha)} = 0.99$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

S = standard deviation of replicate analyses.

^c EDL = estimated detection limit; defined as either MDL (Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11) or a level of compound in a sample yielding a peak in the final extract with signal-to-noise ratio of approximately 5, whichever value is higher.

TABLE 4. LABORATORY PERFORMANCE CHECK SOLUTION

Test	Analyte	Conc. Ng	Requirements
Sensitivity	3-Hydroxycarbofuran	2	Detection of analyte; S/N > 3
Chromatographic performance	Aldicarb sulfoxide	100	0.90 < PGF < 1.1 ^a
Column performance	Methiocarb 4-Bromo-3,5-dimethylphenyl N-methylcarbamate (IS)	20 10	Resolution > 1.0 ^b

^a PGF = peak Gaussian factor. Calculated using the equation:

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where W(1/2) is the peak width at half height in secs. and W(1/10) is the peak width in secs. at tenth height.

^b Resolution between the two peaks as defined by the equation:

$$R = \frac{t}{W}$$

where t is the difference in elution times between the two peaks and W is the average peak width in secs., at the baseline, of the two peaks.

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